Abstract
Multi-well assays based on the Boyden chamber have enabled highly parallel studies of chemotaxis (the directional migration of cells in response to molecular gradients) while direct-viewing approaches have allowed more detailed questions to be asked at low throughput. Boyden-based plates provide a count of cells that pass through a membrane, but no information about cell appearance. In contrast, direct-viewing devices enable the observation of cells during chemotaxis, which allows measurement of many parameters including area, shapes, and location. Here we show automated chemotaxis and cell morphology assays in a 96-well direct-viewing plate. Using only 12,000 primary human neutrophils per datum, we measured dose-dependent stimulation and inhibition of chemotaxis and quantified the effects of inhibitors on cell area and elongation. With 60 parallel conditions and compatibility with existing lab automation, a dramatic increase in throughput compared to previously reported direct viewing approaches is achieved.

Introduction
While modified Boyden chambers in multiwell format are the current standard for highly parallel studies of chemotaxis, direct-viewing approaches, where cells migrate on a horizontal surface, have particular advantages for quantitative studies, such as perturbation of function by drug candidates. The concentration profile experienced by cells in a Boyden chamber is unknown and may be influenced by cells as they traverse membrane pores. In contrast, the concentration gradient produced in direct-viewing devices can be verified using fluorescent dyes. Furthermore, the geometry of modified Boyden chambers is not conducive to microscopical study of cells during chemotaxis, which precludes the use of high content analysis. Existing direct-viewing methods tend to be complicated to run and challenging in terms of throughput. Microfluidic approaches, in turn, have enabled a new level of spatiotemporal control, including defined gradient profiles of various shapes and fast switching between profiles. However, none of the direct-viewing approaches reported to date are suitable for highly parallel studies, the largest number of parallel conditions reported to date is twelve. Microfluidic gradient generators rely on continuous flow to maintain a constant gradient profile over time. Since this demands a unique set of input sources for each experimental condition, highly parallel studies using co-flowing gradients are not feasible. Furthermore, cells in flow can influence experimental results. Devices that do not employ flow must provide means to protect the concentration gradient from disturbances; nano-porous membranes, valved compartments, and fluid-level evaporation are among those that have been used successfully. The objective of our work was to develop a method that would provide the rich information of direct-viewing chemotaxis studies in an automated format with throughput comparable to Boyden-based plates. To this end, a device was developed where the gradient was kept stable by shifting disturbing forces through a low-resistance path. This simple design enabled the construction of large arrays of microfluidic units in which cell and reagent flow could be automated via surface tension-driven passive pumping. Automation allowed highly parallel experiments to be run to study dose-dependent stimulation and inhibition of chemotaxis as well as chemotactic and inhibitory effects on cell morphology.

Direct viewing plate supports spontaneous wick-filling and robust, automated cell patterning

Figure 1: Direct-viewing plate supports wick-filling and cell patterning. a) The plate (bottom view) has 96 microfluidic units located in a rectangular grid pattern with 9 mm x 9 mm spacing. b) Each unit has five components: an attractant port and source channel where a chemokine is provided, a gradient channel connected to the source into which the chemokine diffuses to create a gradient, a cell port (located at the other end of the gradient channel), and a short channel through which flow is driven by capillary action. c) A time-sequence showing wick-filling. d) Since the internal surfaces are hydrophilic, aqueous solutions are drawn into the channels via capillary action. e) As the gradient channel is filled, light at the junction increases in height as the gradient concentration levels increase. f) Once the gradient channel is filled, the cell port is filled up to both ends. g) A schematic representation of a chemotaxis assay. For morphometry assays the cells were used in a different way (see text). h) After wick-filling each unit, cells and culture media were seeded in the cell port and a chemotaxis solution was loaded in the source channel. i) After a period of time the chemokinesis diffusion across the gradient channel and formed a gradient. Chemotactic cells moved into the gradient channel toward increasing concentration. The origin was set to the junction between the well port and gradient channel. The location of the cells was recorded relative to the origin. j) All the beginning of the assay the cells are distributed in a repeatable pattern in the cell port (N=13).

Morphological analysis of chemotactic neutrophils

Figure 4: High content analysis of cell morphology reveals differential effects of inhibitors. Inhibition of neutrophil chemotaxis is associated with inhibitor-specific changes in area and elongation. For this experiment cells were seeded in the gradient channel prior to establishing gradient. Neutrophils were then exposed to parallel conditions with various concentration profiles of IL-8: a) none, b) uniform 0.4 nM, c) uniform 4 nM, or d-g) gradient with 62 nM source. The last condition was used and + (open circle), N=10 for each condition.

Persistent linear gradients are reproducibly formed

Figure 2: The plate provides a repeatable linear gradient. a-d) Characterization of a molecular gradient (Alexafluor555) at 37°C. The graphs show a) gradient profile at 0, 0.5, 2.5 and 10 hours with best fit lines for the last three, b) the slope, and c) the concentration at the origin as determined by least squares line fit for each replicate. Graphs show mean values and standard deviations (N=13). d) The bar graph shows the slope (red) and concentration (green) at 2.5 hours in four different plates run at the same time.

Effects of chemoattractant and inhibitors on neutrophil chemotaxis

Figure 3: Dose-dependent stimulation and inhibition of chemotaxis. a-d) Cell distribution of the end of the assay after 2.5 hour incubation at 0.3 (a), 0.5 (b), 4 (c) and 62 (d) nM IL-8. e) Dose response to migration inhibitors. f-h) Cells in the presence of uniform 4 nM IL-8 and gradient. i) Dose-dependent inhibition of IL-8 mediated chemotaxis (500 nM source). Half-maximum inhibition concentrations (IC50) were found to be 10.8 nM for latrunculin B and 2.32 µM for wortmannin.

Conclusions
- Microfluidic gradient channels arrayed in an SBS/ANSI standard plate were developed.
- The plate enables robust delivery of reagents and cells using off-the-shelf lab automation hardware.
- Chemoattractant gradients are suitable for quantification of polymorphonuclear leukocyte chemotaxis.
- Preliminary assessment of data quality is encouraging for screening applications (Z’=0.39).
- IC50 values of dose response to migration inhibitors was demonstrated.
- Migration on a horizontal surface enables high content analysis such as shape and area.
- Effects of compounds on cell morphology can be quantified to distinguish different classes of inhibitors.

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